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Case report

PD-L1-expressing extranodal diffuse large B-cell lymphoma, NOS with and without *PD-L1* 3'-UTR structural variations

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Immune evasion mediated by PD-L1 plays an important role in the development of B-cell malignancies. However, PD-L1 expression is infrequently observed in tumor cells of extranodal diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS). Other than copy number alterations, PD-L1 is aberrantly upregulated by structural variations in the 3′-UTR of *PD-L1*. We report four cases with PD-L1 expression on tumor cells, including two with structural variations in the 3′-UTR of *PD-L1* and two without. Our report demonstrates the presence of a small number of "immune evasion-type" extranodal DLBCL, NOS cases.

Keywords: PD-L1, extranodal DLBCL

INTRODUCTION

Immune evasion mediated by programmed cell death 1 (PD-1; also known as PDCD1) and programmed cell death 1 ligand 1 (PD-L1; also known as CD274) plays an important role in the development of many types of cancer. PD-L1 expression on tumor cells inhibits cytotoxic T-cell activities and protects tumor cells from eradication by the immune system.[1] Among B-cell malignancies, it has been well documented that the PD-1/PD-L1 axis plays a role in Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. In contrast, PD-L1 expression is much less frequently observed in tumor cells of extranodal diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS).[2] Recently, our group demonstrated that PD-L1 (detected with clone SP142 antibodies) was expressed on tumor cells in only four of 87 cases of extranodal DLBCL, NOS and in three of 174 cases of gastrointestinal DLBCL, NOS.[2,3]

The molecular mechanism of PD-L1 upregulation is related to disease type. In solid neoplasms, *PD-L1* is transcriptionally upregulated as a result of intercellular interactions between tumor cells and inflammatory cells in the tumor microenvironment.[4] On the other hand, in B-cell malignancies, *PD-L1* expression is frequently upregulated as a result of genomic aberrations. Nearly 100% of Hodgkin lymphomas and 75% of primary mediastinal large B-cell lymphomas harbor a copy number alteration (CNA) in 9p24.1, where *PD-L1* resides.[5,6] In addition, Kataoka *et al*. demonstrated that structural variations in the 3'-untranslated region (3′-UTR) of *PD-L1* upregulate *PD-L1* transcripts. Structural variations of the *PD-L1* 3′-UTR were found in 8% of DLBCLs.[7] However, the clinical significance of disruption in the *PD-L1* 3′-UTR in lymphomas remains unknown.

We investigated whether three previously described cases of extranodal DLBCL, NOS with PD-L1 expression on tumor cells[2] and an additional case of intestinal DLBCL with PD-L1 expression[3,8] harbor *PD-L1* 3′-UTR structural variations. These cases highlighted the role of the PD-1/PD-L1 axis in the pathogenesis of a subset of extranodal DLBCL, NOS cases.

CLINICAL SUMMARY

The clinical and pathological findings of these four patients were previously documented in separate reports; they are described in detail and summarized in Table 1.

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Table 1. Characteristics of patients

	#1	#2	#3	#4
Clinical characteristics				
Age	63	73	59	70
Sex	male	male	female	male
PS	0	2	0	3
IPI	LI	HI	LI	HI
Ann Arbor Stage	NA	I	IV	I
Lugano Stage	IV	NA	NA	NA
B symptoms	-	-	+	+
Hepatomegaly	-	-	-	-
Splenomegaly	-	-	+	-
Lymphadenopathy	+	-	-	-
Neurological symptoms	-	-	-	-
Involved site	mediastinum, cervical LN, inguinal LN, lung, stomach, ileocecum, adrenal gland	pelvic cavity	adrenal gland	adrenal gland and bone marrow
Laboratory data				
WBC	9400	9400	4300	7800
Hb	9.7	9.8	8.8	11.8
Plt	17.2	17.2	0.9	11.4
LDH	196	over	over	over
Alb	1.8	2.7	3.6	3.5
CRP	0.7	16.1	3.0	7.2
s-IL2R	2760	4520	1590	3070
Treatment	R-CHOP	R-CHOP+IT	R-CHOP+IT	none
Initial response	CR, relapse(5)	CR	CR	NA
Outcome	DD(12)	AWD(36)	AWD(12)	DD(0.3)
Immunohistochemical findings				
CD5	-	-	-	-
CD10	-	-	-	-
CD20	+	+	+	+
BCL2	+	+	+	+
BCL6	-	+	+	+
MUM1	+	+	+	+
Hans' criteria	non-GCB	non-GCB	non-GCB	non-GCB
EBER	-	-	-	-
PD-L1 (%)	>90	>90	30	10

PS, performance status; LI, low–intermediate; HI, high–intermediate; IPI, international prognostic index; LN, lymph node; WBC, white blood cells, Hb, hemoglobin; Plt, platelets; LDH, lactate dehydrogenase; Alb, albumin; CRP, C-reactive protein; s-IL2R, soluble interleukin-2 receptor; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone; IT, intrathecal chemotherapy; CR, complete remission; DD, died of the disease; AWD, alive without disease; EBER, Epstein-Barr virus (EBV)-encoded small RNA, PD-L1, programmed death-ligand 1.

Case 1 was previously described by Ishikawa *et al*.,[3] and Cases 2-4 were previously described by Suzuki *et al*.[2]

Case 1

A 63-year-old male initially presented with abdominal pain with no underlying disease or relevant history. Laboratory findings were within the normal range, except the following were noted: anemia, high soluble interleukin-2 receptor, slightly high C-reactive protein, and low albumin. He was diagnosed with ileus and underwent ileocecal resection. The surgical excision specimen revealed an ulcerative tumor in the ileocecum (Figure 1a), which was pathologically diagnosed as DLBCL, NOS. Positron emission tomography/computed tomography (PET/CT) revealed the involvement of systemic lymph nodes, including cervical, mediastinal, paraaortic, and inguinal lymph nodes. Moreover, multiple organs were involved, including the lung, stomach, and adrenal glands. The tumor was categorized as Lugano stage IV.

To treat DLBCL, the patient underwent chemotherapy with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). The patient achieved complete remission 5 months after chemotherapy initiation. Two

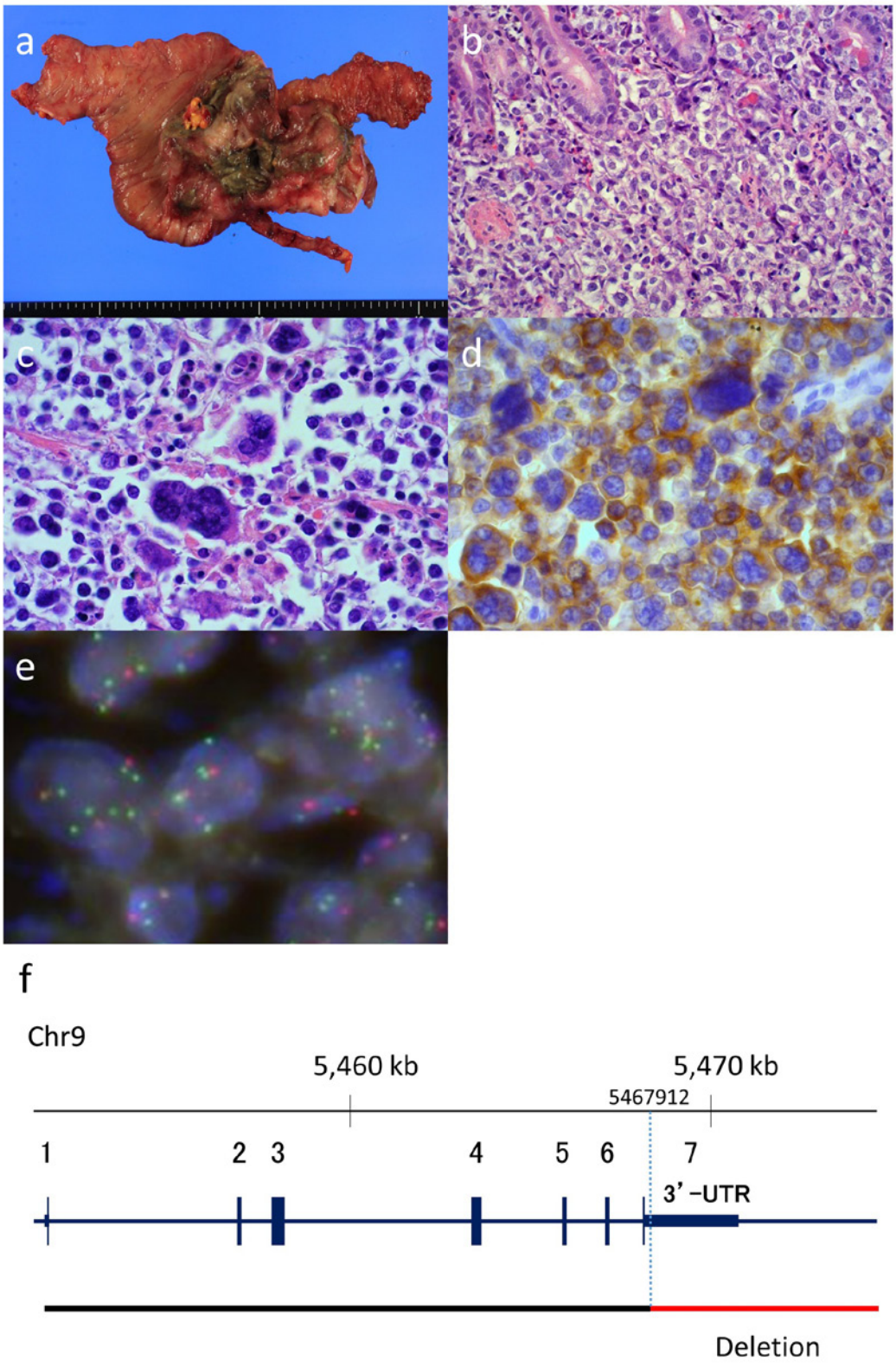

Fig. 1. Histology and genomic features of neoplastic PD-L1^{+} extranodal DLBCL (Case 1)
(***a***) Macroscopic image of the surgically excised specimen. An ulcerative tumor formed in the ileocecum. (***b***) Micrograph of the mucosa shows diffuse proliferation of atypical lymphoid cells. (***c***) Micrograph of a tumor section shows scattered binucleated and multinucleated cells. (***d***) Micrograph of tumor cells shows that multinucleated cells express PD-L1. (***e***) Fluorescence micrograph of tumor cells labeled with fluorescence *in situ* hybridization shows multiple green signals, which indicate amplification of the *PD-L1* locus. (***f***) The structural variation involving the 3'region of the *PD-L1* gene. The deleted region (*red*) did not affect the coding sequence of *PD-L1*.

months after the achievement of complete remission, relapse of DLBCL was detected by stomach biopsy. He received salvage therapy, which included cyclophosphamide, cytosine arabinoside, etoposide, and dexamethasone, and radiation therapy. However, he had progressive disease and died of the disease 12 months after the initial diagnosis.

Case 2

A 73-year-old male, with no underlying disease, initially presented with lower leg pain. Laboratory findings were within the normal range, except the following: anemia, increases in soluble interleukin-2 receptor, C-reactive protein, and lactate dehydrogenase, and low albumin. CT revealed a solitary tumor mass in the pelvic cavity, with a diameter of 75×45 mm. Needle biopsy was performed and the pathological diagnosis was DLBCL, NOS. No systemic lymph node swelling or extranodal involvement, other than the pelvic mass, was detected on PET/CT. After the diagnosis, he received chemotherapy with R-CHOP, and subsequently, intrathecal chemotherapy and irradiation (total 30 Gy). He achieved complete remission 5 months after the initial therapy and he has survived without disease for 36 months after the initial diagnosis.

Case 3

A 59-year-old female, with no underlying disease, had fever of unknown origin. PET/CT revealed bilateral adrenal masses. She also had an enlarged spleen, although increased FDG uptake was not observed in the spleen. Laboratory findings included anemia, thrombocytopenia, and increases in C-reactive protein and lactate dehydrogenase. The diagnosis of DLBCL was made based on biopsy of the adrenal gland. After the diagnosis, she received R-CHOP and intrathecal chemotherapy, and achieved complete remission. She has survived without disease for 12 months after the initial diagnosis.

Case 4

A 70-year-old male with a history of intestinal pneumonia and myocardial infarction had rapidly growing bilateral adrenal masses on CT. He had dyspnea, but CT demonstrated no abnormalities in the lung to explain the symptoms. Laboratory findings included anemia, thrombocytopenia, and increases in C-reactive protein, lactate dehydrogenase, and serum soluble interleukein-2 receptor. The diagnosis of DLBCL was made based on biopsy of the adrenal gland, and bone marrow biopsy revealed bone marrow involvement by DLBCL. The patient did not receive any therapy for DLBCL and died 10 days after the initial diagnosis.

PATHOLOGICAL FINDINGS

Case 1

Histologically, the ileocecal tumor exhibited diffuse proliferation of atypical large lymphoid cells with a polymorphous background of small lymphocytes, neutrophils, and histiocytes (Figure 1b). Focally, large multinucleated giant cells were observed (Figure 1c). The neoplastic cells, including the multinucleated giant cells, were positive for CD20, BCL2, and MUM1, and negative for CD5, CD10, BCL6, and EBER. Nearly 100% of the neoplastic cells were positive on staining for PD-L1 using clone SP142 antibodies (Figure 1d). Detection of *PD-L1* genetic alterations was performed as previously described.[9] Briefly, CNA of *PD-L1* was assessed by dual-color FISH analysis using a SPEC CD274, PDCD1LG2/CEN9 Dual Color Probe (Zytovision, Bremerhaven, Germany). The FISH analysis revealed *PD-L1* gene amplification (Figure 1e). Structural variations affecting *PD-L1* were explored using targeted-capture sequencing with a custom SureSelect library (Agilent Technologies, Santa Clara, CA, USA), which can capture the entire sequence of the *PD-L1*,[9] revealing a deletion in the 3′-UTR of the *PD-L1* gene (Figure 1f).

Case 2

Histologically, the pelvic cavity tumor mass demonstrated monomorphic atypical large lymphoid cell proliferation with marked apoptosis and mitotic figures (Figure 2a). The neoplastic cells had an immunophenotype similar to that observed in Case 1, except that they had BCL6positive staining. Nearly 100% of the neoplastic cells exhibited membranous staining of CD20 and PD-L1 (Figure 2b, c). FISH analysis did not detect CNA in the *PD-L1* locus. However, it demonstrated complex structural variations in the 3′-UTR of *PD-L1*, with an intrachromosomal inversion and an interchromosomal translocation, both structural variations shearing the identical breakpoint (Figure 2d).

In both Cases 1 and 2, the structural variations did not affect the *PD-L1* coding sequence. No other gene mutations were detected in the coding sequence of *PD-L1*.

Cases 3 and 4

Both cases were characterized by the diffuse proliferation of monomorphic large transformed B-cells with a centroblastic (Case 3) or immunoblastic (Case 4) appearance (Figure 3a, b). In contrast to Cases 1 and 2, only a small sub-population of tumor cells expressed PD-L1 in Cases 3 and 4 (Figure 3c, d). Of note, both Cases 3 and 4 had a partially intravascular pattern, and PD-L1 expression was observed in the intravascular tumor cells (Figure 3e, f). No *PD-L1*-associated CNA or structural variations were detected in Cases 3 and 4 by FISH analysis or targeted-capture sequencing.

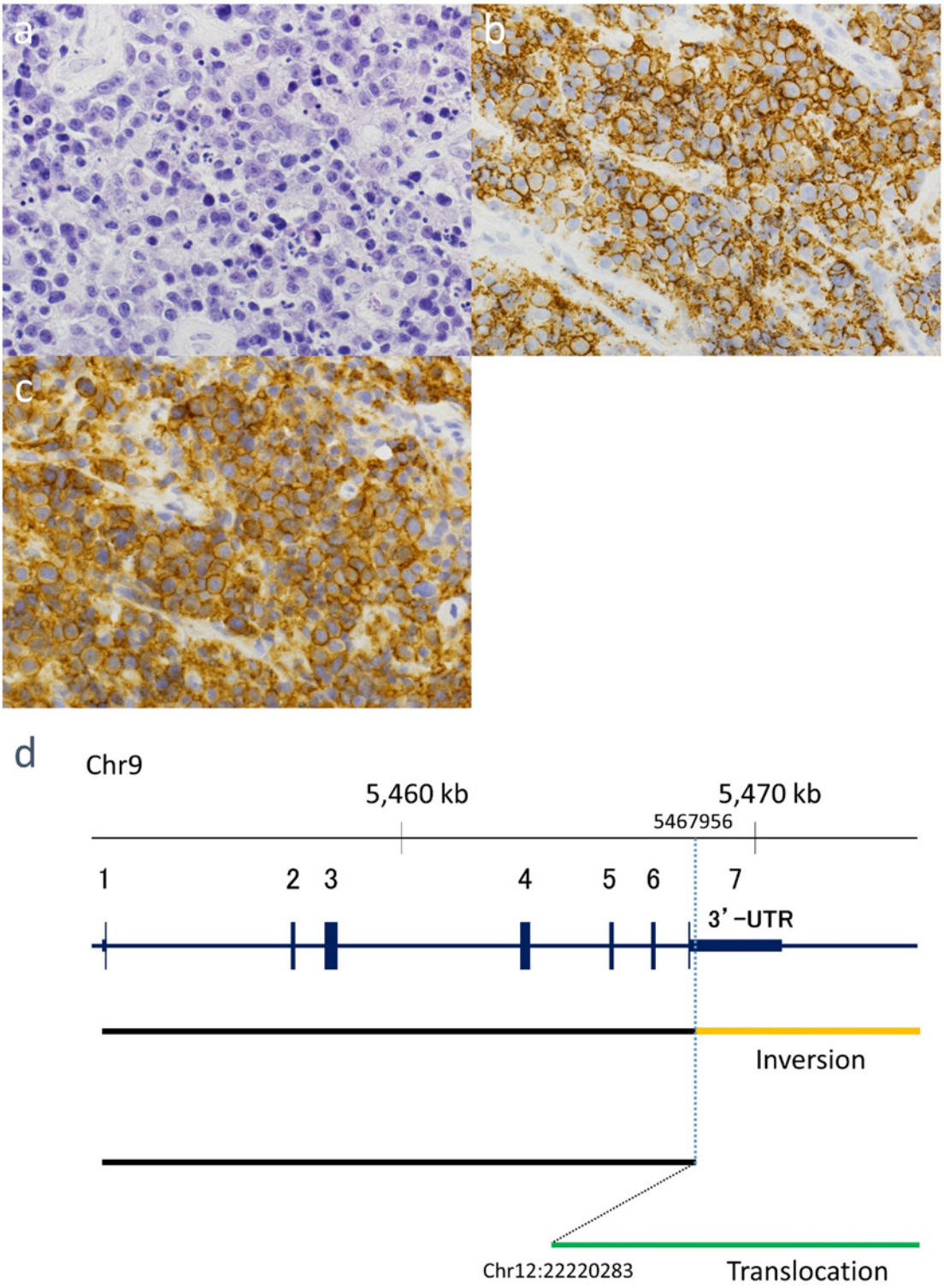

Fig. 2. Histology and genomic features of neoplastic PD-L1^{+} extranodal DLBCL (Case 2)
(***a***) Micrograph of a pelvic tumor section shows diffuse proliferation of large atypical lymphoid cells, admixed with polymorphous inflammatory cells. (***b***, ***c***) Micrographs of tumor cells show membranous expression of (***b***) CD20 and (***c***) PD-L1. (***d***) Structural variations (SVs) involving the 3'region of the *PD-L1* gene. At the same breakpoint (chr9:5467956), we observed (*top*) an intrachromosomal inversion (*yellow*) and (*bottom*) a translocation (*green*) between chromosomes 9 and 12. The SVs did not affect the coding sequence of *PD-L1*.

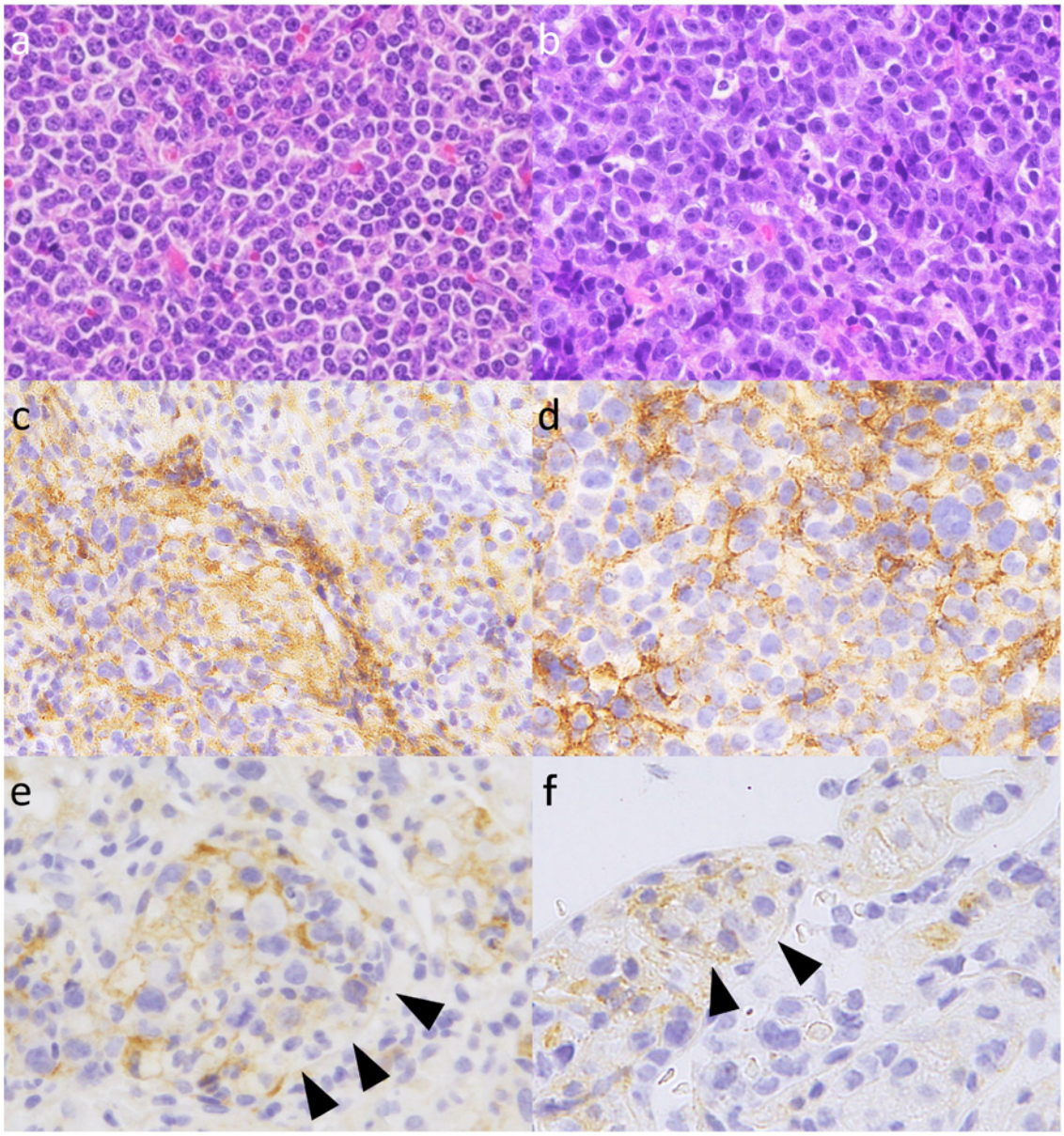

Fig. 3. Histology of PD-L1$^+$ extranodal DLBCL without *PD-L1*-associated genetic alterations
Micrograph of diffuse tumor cell proliferations in the adrenal gland in Cases 3 (***a***) and 4 (***b***). Only a small population of tumor cells expressed PD-L1 in both Cases 3 (***c***) and 4 (***d***). Both Cases 3 and 4 were characterized by an intravascular pattern in which tumor cells expressed PD-L1 (***e***, ***f***, black arrowheads).

DISCUSSION

Tumor-cell immune evasion, mediated by the PD-1/PD-L1 axis, is believed to play a central role in the pathogenesis of a subset of B-cell lymphomas. The role of the PD-1/PD-L1 axis has been particularly emphasized in Hodgkin lymphoma and primary mediastinal large B-cell lymphoma, where CNA in *PD-L1* was reported in nearly 100% and 75% of cases, respectively.[5,6] In contrast, neoplastic PD-L1 expression has been observed less frequently in DLBCL, NOS. Our group recently reported neoplastic PD-L1 expression in only 1.7% of extranodal DLBCL, NOS cases.[2] In the present study, we described the occurrence of a small number of "immune evasion-type" extranodal DLBCL, NOS cases, where structural variations caused the upregulation of *PD-L1*.

In Case 2, DLBCL, NOS was characterized by exclusive extranodal involvement. However, the primary site of origin remained uncertain in Case 1 because this patient had multiple extranodal and nodal lesions at the time of diagnosis. However, the main tumor mass in Case 1 was an ileocecal tumor, thus it is plausible that tumor cells preferentially proliferated in the ileocecum.

Previous studies described several characteristics of neoplastic PD-L1$^+$ extranodal DLBCL, NOS.[2] Intravascular patterns are frequently observed, which suggested that the pathogenesis of neoplastic PD-L1$^+$ extranodal DLBCL, NOS is linked to the pathogenesis of intravascular large B-cell lymphoma (IVL).[2] Recently, Shimada *et al*. reported that

structural variations that affected the 3′-UTR of *PD-L1* are frequently present in IVL.[10] However, although both of our cases had structural variations in *PD-L1*, neither exhibited an intravascular pattern.

Cases 1, 3, and 4 also featured adrenal gland involvement, which is another characteristic of neoplastic PD-L1$^+$ extranodal DLBCL, NOS. Of note, the adrenal gland has been described as an immune-sanctuary site where tumor cells can evade immune checkpoint therapy.[11] However, PD-L1 is presumed to be upregulated on tumor cells to evade tumor-specific T cells; therefore, it is paradoxical for tumor cells that express PD-L1 to preferentially propagate in immune-sanctuary sites, where they will encounter a limited anti-tumor immune reaction.[11] However, activation of the PD-1/PD-L1 axis is a hallmark of DLBCL with involvement of other immune-sanctuary sites. Indeed, CNA in *PD-L1* is a common genetic aberration among DLBCLs in the central nervous system or testes.[12] Future studies that focus on interactions between the microenvironment and tumor cells are needed to elucidate the pathogenesis of neoplastic PD-L1$^+$ extranodal DLBCL, NOS.

In both cases with *PD-L1* structural variations, the breakpoints of the structural variations were located approximately 100 bp downstream of the *PD-L1* stop codon. Of note, the breakpoint in Case 1 was within the WRCY motif of PD-L1, which is susceptible to somatic hypermutation or gene recombination induced by the activation-induced deaminase (AID).[13] Therefore, some structural variations that affect *PD-L1* may be induced by AID in germinal centers. In support of this hypothesis, *PD-L1* transcription was reported to be upregulated in germinal centers,[14] and upregulated transcription was demonstrated to be associated with increased susceptibility to AID-mediated gene editing.[13] Furthermore, the co-occurrence of *PD-L1* structural variations and mutations in AID-target genes, such as *PIM1* mutations, were frequently identified in IVL.[10] This suggested that somatic hypermutations are associated with PD-L1 structural variations, which may provide new insight into the pathogenesis of neoplastic PD-L1$^+$ extranodal DLBCL, NOS.

In comparison with PD-L1$^+$ extranodal DLBCL, NOS with *PD-L1* 3′-UTR structural variations (Cases 1, 2) and without *PD-L1* 3′-UTR structural variations (Cases 3, 4), tumor cells in Cases 1 and 2 uniformly expressed PD-L1, whereas tumor cells in Cases 3 and 4 had heterogeneous PD-L1 expression. This difference may reflect the mechanism of PD-L1 upregulation. Although *PD-L1* 3′-UTR structural variations can lead to constitutive *PD-L1* upregulation,[7] PD-L1 expression induced by intracellular signaling, such as the IFN-γ pathway, can be transient, leading to intratumoral heterogeneity of PD-L1 expression.[15] The difference in mechanisms leading to PD-L1 expression may have clinical relevance, as temporal changes in PD-L1 expression on tumor cells have been suggested to be one of the mechanisms of resistance to immune checkpoint therapy.[16]

In previous reports, structural variations in *PD-L1* frequently affected exon 6 or 7, which disrupted the intracellular domain of PD-L1 with the extracellular domain unaffected. This disruption altered the binding characteristics of PD-L1 to anti-PD-L1 antibodies. This alteration can explain the relatively low sensitivity of the SP142 antibody, which recognizes the intracellular domain of PD-L1.[1,17] Consistent with this explanation, in both cases described in this report, exon 7 was retained and binding to the SP142 antibody was preserved. Our group previously revealed that positive binding to the SP142 antibody has clinical implications in several subtypes of DLBCL. For example, positive SP142 binding was a poor prognostic factor in gastrointestinal DLBCL, IVL, adrenal gland DLBCL (Kawano *et al*. in preparation), and EBV$^+$DLBCL, NOS.[1,3,17] In addition to its immunomodulatory function, which is mediated by the extracellular domain of PD-L1, the intracellular domain engages in cell survival via STAT3 activation. This signaling pathway may be involved in the poor prognosis of SP142-positive DLBCL.[18] Large studies are required to investigate whether the structural variations in *PD-L1* affect clinical outcomes.

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AUTHOR CONTRIBUTIONS

T.T. collected data, analyzed data, interpreted data, and wrote the manuscript. E.I., Y.S., Y.K., A.S., K.K., and S.N. collected data, analyzed data, interpreted data, and critically reviewed the manuscript. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have no significant relationships with or financial interests in any commercial companies pertaining to this article. This study was performed after institutional review board approval by the Aichi Medical University ethical review board.

REFERENCES

1 Sakakibara A, Kohno K, Ishikawa E, *et al*. Age-related EBV-associated B-cell lymphoproliferative disorders and other EBV + lymphoproliferative diseases: New insights into immune escape and immunodeficiency through staining with anti-PD-L1 antibody clone SP142. Pathol Int. 2020; 70: 481-492.

2 Suzuki Y, Sakakibara A, Shimada K, *et al*. Immune evasion-related extranodal large B-cell lymphoma: A report of six patients with neoplastic PD-L1-positive extranodal diffuse large B-cell lymphoma. Pathol Int. 2019; 69: 13-20.

3 Ishikawa E, Nakamura M, Shimada K, *et al*. Prognostic impact of PD-L1 expression in primary gastric and intestinal diffuse large B-cell lymphoma. J Gastroenterol. 2020; 55: 39-50.

4 Chen J, Jiang CC, Jin L, Zhang XD. Regulation of PD-L1: a

novel role of pro-survival signalling in cancer. Ann Oncol. 2016; 27: 409-416.
5 Swerdlow SH, Campo E, Harris NL, *et al*. WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. 4th ed, Lyon, IARC, World Health Organization. 2017.
6 Younes A, Santoro A, Shipp M, *et al*. Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. Lancet Oncol. 2016; 17: 1283-1294.
7 Kataoka K, Shiraishi Y, Takeda Y, *et al*. Aberrant PD-L1 expression through 3′-UTR disruption in multiple cancers. Nature. 2016; 534: 402-406.
8 Ishikawa E, Kato S, Shimada K, *et al*. Clinicopathological analysis of primary intestinal diffuse large B-cell lymphoma: Prognostic evaluation of CD5, PD-L1, and Epstein-Barr virus on tumor cells. Cancer Med. 2018; 7: 6051-6063.
9 Yamashita D, Shimada K, Kohno K, *et al*. PD-L1 expression on tumor or stromal cells of nodal cytotoxic T-cell lymphoma: A clinicopathological study of 50 cases. Pathol Int. 2020; 70: 513-522.
10 Shimada K, Yoshida K, Suzuki Y, *et al*. Frequent genetic alterations in immune checkpoint-related genes in intravascular large B-cell lymphoma. Blood. 2021; 137: 1491-1502.
11 Nguyen MC, Shah MH, Liebner DA, *et al*. The adrenal gland as a sanctuary site of metastases after pembrolizumab treatment: A case series. J Natl Compr Canc Netw. 2018; 16: 1279-1283.
12 Chapuy B, Roemer MGM, Stewart C, *et al*. Targetable genetic features of primary testicular and primary central nervous system lymphomas. Blood. 2016; 127: 869-881.
13 Casellas R, Basu U, Yewdell WT, *et al*. Mutations, kataegis and translocations in B cells: understanding AID promiscuous activity. Nat Rev Immunol. 2016; 16: 164-176.
14 Good-Jacobson KL, Szumilas CG, Chen L, *et al*. PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. Nat Immunol. 2010; 11: 535-542.
15 Noguchi T, Ward JP, Gubin MM, *et al*. Temporally distinct PD-L1 expression by tumor and host cells contributes to immune escape. Cancer Immunol Res. 2017; 5: 106-117.
16 Takahashi T, Tateishi A, Bychkov A, Fukuoka J. Remarkable alteration of PD-L1 expression after immune checkpoint therapy in patients with non-small-cell lung cancer: Two autopsy case reports. Int J Mol Sci. 2019; 20: 2578.
17 Takahara T, Satou A, Ishikawa E, *et al*. Clinicopathological analysis of neoplastic PD-L1-positive EBV(+) diffuse large B cell lymphoma, not otherwise specified, in a Japanese cohort. Virchows Arch. 2021; 478: 541-552
18 Escors D, Gato-Cañas M, Zuazo M, *et al*. The intracellular signalosome of PD-L1 in cancer cells. Signal Transduct Target Ther. 2018; 3: 26.